

The effects of sulphuric acid (pH3) on roots and mycorrhizas of *Pinus sylvestris*

J. DIGHTON*, R.A. SKEFFINGTON** and K.A. BROWN**

* Institute of Terrestrial Ecology, Merlewood Research Station,
Grange-over-Sands, Cumbria LA11 6JU, Great Britain

** CEGB, Central Electricity Research Laboratories,
Kelvin Avenue, Leatherhead, Surrey KT22 7SE, Great Britain

INTRODUCTION

This paper reports the effects of 5 years application of simulated acid rain (at pH 3) on root biomass and mycorrhizal associations of Scots pine growing in monolith lysimeters. Results of studies on the effects of acid treatment on the above ground tree growth, plant and soil chemistry and soil microbiology will be published elsewhere.

LYSIMETER AND SAMPLING DETAILS

Undisturbed cores of brown podzolic soil from a Lower Greensand series were taken in monolith lysimeters, 0.8 m diam. x 1.35 m deep (RIPPON, 1980). A synthetic rain solution (pH 5.6), similar in composition to precipitation at Birkenes, S. Norway, was applied at 1500 mm year⁻¹ for 5 years to two control lysimeters. The two acid-treated lysimeters received 'rain' solution acidified to pH 3 with sulphuric acid. Full details are given by RIPPON *et al.* (1985).

Roots of Scots pine (*Pinus sylvestris* L.) saplings (3 years old), planted at a density of about 60/lysimeter, were extracted from 3 cm depth horizons from soil, taken on a stratified random basis, from 6 identically located segments in each lysimeter, using a 2.5 cm diameter soil corer to a depth of 30 cm. Fine root length was estimated using a modified line intersect method (NEWMAN, 1966; TENNANT, 1975) with the aid of a Quantimet image analyser. The total number of root tips in each of 6 mycorrhizal categories was counted for each sample. Characters used to classify mycorrhizal types followed those of DOMINIK (1969) (Table 2).

Samples of mycorrhizas were sectioned by hand or after resin embedding (L.R. White resin) and examined under the light microscope for evidence of reduced sheath thickness, abnormal Hartig net development or obvious damage to the cortex and endodermis.

RESULTS

Mean fine (≤ 1 mm diameter) and coarse (> 1 mm diameter) root lengths and the number of root tips occurring in each 3 cm soil depth horizon for the acid and control lysimeters are given in Table 1. Each value is the mean of

12 samples, 6 being taken from each of the replicate lysimeters in each treatment.

Table 1. Composite table of means of fine and coarse root length (cm) and number of root tips for each depth horizon taken from the control and acid lysimeters. Overall standard errors are given.

Soil depth horizon	Fine root length (≤ 1 mm)			Coarse root length (> 1 mm)			No. of root tips		
	Con	Acid	SE	Con	Acid	SE	Con	Acid	SE
Litter	88	83	10.4	0.0	0.0	0.0	629	274	65.9
0-3 cm	154	130	37.6	3.0	2.8	0.4	983	390	52.6
3-6 cm	58	53	6.2	0.6	1.7	0.4	282	173	37.0
6-9 cm	40	40	4.7	1.2	1.3	0.3	206	150	21.6
9-12 cm	25	20	3.3	1.1	1.2	0.3	101	53	18.5
12-15 cm	15	10	3.0	0.4	1.2	0.3	66	29	9.5
15-18 cm	5	6	1.4	0.0	0.8	0.2	20	21	6.8
18-21 cm	4	7	1.2	0.3	0.6	0.2	11	25	13.0
21-24 cm	9	14	3.1	0.2	0.3	0.2	26	36	8.3
24-27 cm	9	11	2.0	0.0	1.0	0.3	27	30	9.5
27-30 cm	14	4	2.3	0.0	0.2	0.1	36	14	4.1

Root length differed little between the two treatments. The overall standard errors (Table 1) show that fine root length differed significantly only in the 18-21 cm and 27-30 cm horizons. There is, however, a consistent trend for greater coarse root length in soil from the acid lysimeters (significant differences at depths 3-6, 12-15, 15-18 and 24-27 cm).

The number of root tips, however, is significantly greater in the control lysimeter for depths down to 5 cm. This significant increase in root branching is attributed mainly to two coralloid mycorrhizal morphs, Types B and F and also, partly, to an increase in Type A (Table 2; Fig. 1). These differences are statistically significant only in the top 6 cm of soil.

Table 2. Mean number of root tips ($\pm$ S.E.) per core, over all depth horizons, of each mycorrhizal category, between the two lysimeter treatments.

Mycorrhizal type	Dominik's genus	Number of root tips per core	
		Control	Acid
Type A	Bf	638 $\pm$ 58.4	491 $\pm$ 70.1
Type B	Hd	491 $\pm$ 149.9	14 $\pm$ 3.9
Type C	Bb	293 $\pm$ 37.6	264 $\pm$ 79.2
Type D	Bb	29 $\pm$ 10.4	42 $\pm$ 17.1
Type E	Kb	114 $\pm$ 29.7	101 $\pm$ 27.0
Type F	Cf	149 $\pm$ 41.9	7 $\pm$ 3.8
Non mycorrhizal		13 $\pm$ 3.0	10 $\pm$ 1.5

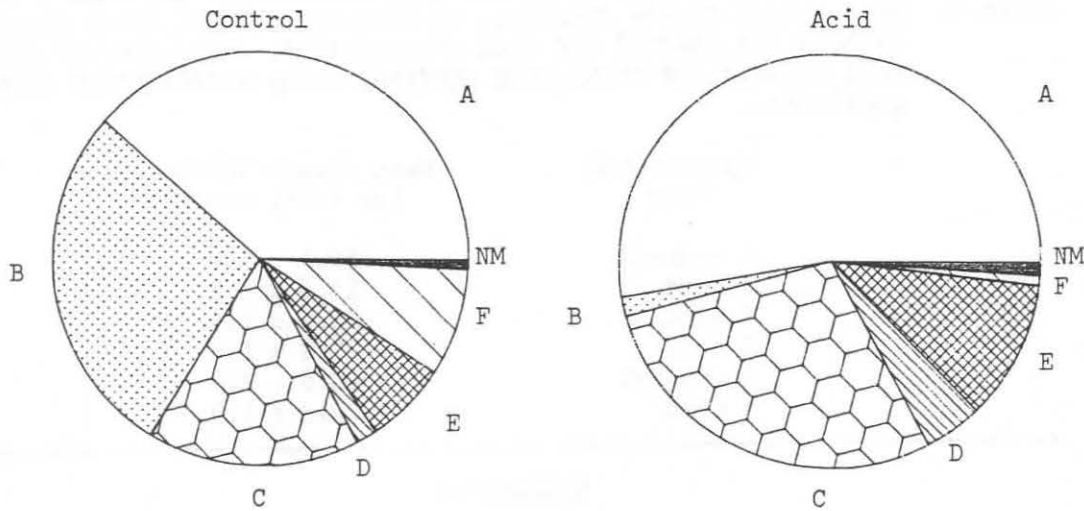


Figure 1. Effects of acid treatment on the proportional numbers of root tips with different mycorrhizal associations (A to F; NM = non-mycorrhizal)

The best fit for the average number of root tips of each mycorrhizal association with increasing soil depth is given by the power law equation $Y = a.X^b$ (where Y = tip number, X = lower depth of a horizon). The parameters of the fitted regressions are given in Table 3 which show elevated values for parameter a in mycorrhizal types A, B and F in control lysimeters.

Table 3. Parameters of the power law equations for the regression of number of root tips of each mycorrhizal association per lower limit of horizon depth.

Mycorrhizal Association	Control			Acid		
	a	b	r ²	a	b	r ²
A	1536	-1.515	0.801	876	-1.832	0.860
B	5123	-2.421	0.854	17	-1.023	0.840
C	899	-1.561	0.917	833	-1.593	0.866
D	105	-1.724	0.834	102	-1.590	0.597
E	502	-1.944	0.546	259	-1.529	0.691
F	1848	-2.332	0.754	19	-1.348	0.802
NM	41	-1.601	0.802	14	-1.220	0.715
Total	6873	-1.765	0.871	2333	-1.483	0.891
(NM = non mycorrhizal)						

The gross morphological characters of the mycorrhizas are given by DIGHTON (in preparation), but it is of interest to note that both mycorrhizal types B and F are characterized by large quantities of extra-radical hyphae and indications of strand development.

There were no obvious signs of abnormality in the development of sheath and Hartig net, as a result of acid treatment. Table 4 compares sheath thickness of mycorrhizas common to both acid and control lysimeters,

showing no detectable differences. Similarly, no damage to the endodermal region of these roots was observed as a result of acid treatment.

Table 4. Mean fungal sheath thickness of like mycorrhizal types occurring in both the control and acid treated soil. Measurements were made on hand cut transverse sections using a calibrated eyepiece graticule.

Mycorrhizal type	Mean sheath thickness ($\mu\text{m} \pm \text{SE}$, $n = 10$)
A control	10.5 ± 1.0
A acid	10.6 ± 0.8
C control	21.4 ± 1.5
C acid	21.0 ± 1.6
E control	20.8 ± 2.5
E acid	22.3 ± 1.9

DISCUSSION

The major changes in soil chemistry as a result of acid treatment were a pH drop of 0.2-0.3 units (depending on depth) and increased leaching of calcium, magnesium and manganese ions in the top 30 cm of the profile (as previously observed in lysimeters without trees (BROWN, 1983)). This acidification, however, had little effect on the length of Scots pine fine root material in the acid treated soil. In the litter and upper layers of soil, significantly more root branching occurred per unit root length in the control than acid treated lysimeters.

The increase in fine root branching of control lysimeters was particularly related to two mycorrhizal morphs (Types B and F): these were characterized by extensive extra-radical hyphal development. The significantly reduced development of these types in acid-treated soil may have been an indirect effect of reduced soil pH in increasing the solubility of toxic elements such as aluminium and manganese. Soil solution data show an increase in concentration of aluminium and manganese in the acid treated lysimeters. FIRESTONE *et al.* (1983) showed that acid induced mobilization of aluminium and manganese significantly reduced growth of *Aspergillus* sp. Similarly, THOMPSON and MEDVE (1983) demonstrated reduced growth of the mycorrhizal species *Suillus luteus*, *Cenococcum graniforme* and *Thelephora terrestris* in culture with increasing levels of aluminium and, to a lesser extent, with manganese. They also showed these fungi to have differing levels of tolerance to aluminium toxicity with *Suillus* being more tolerant than *Cenococcum*. If the development of mycorrhizal types B and F is dependent upon the production of extensive hyphal outgrowths, the potential increased availability of toxic metal elements may account for their virtual absence in acid-treated lysimeters.

It seems unlikely that a drop in pH *per se* would have a significant effect in selecting different mycorrhizal associates. Indeed, MARX and ZAK (1965) suggest that an increase in pH is probably more inhibitory to mycorrhizal success than the converse.

A change in the mycorrhizal species composition on Scots pine saplings has been noted as the outcome of prolonged acid treatment under these experimental conditions. An increase in dead or dying roots, as observed

by SOBOTKA (1964) on older Norway spruce trees, however, has not been noted here. As the health of like mycorrhizal types occurring under both treatments does not seem to be affected (Table 4), it may be assumed that the mycorrhizal components of the acid treated root system, although reduced in species abundance, has developed to optimize conditions prevalent under soil conditions resulting from this treatment.

The acid treatment used in this experiment contained 10-20 times the concentration of H_3O^+ as acid rain at pH 4-4.3 (typical of northern Europe) and deposited a flux of H_3O^+ ($1500 \text{ meq m}^{-2} \text{ year}^{-1}$) at least 2.4 times greater than in throughfall under pines at a site receiving high inputs of acid deposition (Brown, 1983). The effects of the treatment would, therefore be expected to be substantially greater than those which may occur in areas receiving smaller and more dilute inputs of atmospheric acidity.

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